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Research paper

Sustained intrathecal therapeutic protein delivery using genetically transduced tissue implants in a freely moving rat model

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ABSTRACT

Systemic delivery of therapeutic proteins to the central nervous system (CNS) is challenging because of the blood-brain barrier restrictions. Direct intrathecal delivery is possible but does not produce stable concentrations. We are proposing an alternative approach for localized delivery into the CNS based on the Transduced Autologous Restorative Gene Therapy (TARGT) system. This system was previously developed using a gene therapy approach with dermal tissue implants. Lewis rat dermal tissue was transduced to secrete human EPO (hEPO). TARGT viability and function were retained following cryopreservation. Upon implantation into the rat cisterna magna, a mild inflammatory response was observed at the TARGT-brain interface throughout 21-day implantation. hEPO expression was verified immunohistochemically and by secreted levels in cerebrospinal fluid (CSF), serum, and *in vitro* post explant. Detectable CSF hEPO levels were maintained during the study. Serum hEPO levels were similar to rat and human basal serum levels. *In vitro*, the highest hEPO concentration was observed on day 1 post-explant culture and then remained constant for over 21 days. Prolonged incubation within the cisterna magna had no negative impact on TARGT hEPO secretion. These promising results suggest that TARGTs could be utilized for targeted delivery of therapeutic proteins to the CNS.

1. Introduction

Therapeutic protein delivery to the central nervous system (CNS) is challenging because of the restrictions imposed by the blood-brain barrier (BBB). This barrier permits only transport of molecules essential for brain function and is highly selective and almost impermeable to drug transport. Direct intrathecal delivery of therapeutic agents into the CNS is possible, but requires an implanted reservoir or repeated external access of a device, like an Ommaya reservoir. Moreover, these approaches do not produce stable concentrations. Alternatively, ultrasound has been used to deliver chemotherapeutic drugs by disrupting BBB tight junctions (Beccaria et al., 2016), but the ultrasound parameters must be optimized to avoid tissue damage. Additionally, highly targeted drug delivery is being developed with liposomal nanocarriers that cross the BBB to deliver therapeutic agents to a specific site (Koo et al., 2006). However, the liposome design must be modulated for each application as a universal liposomal formulation does not exist.

We are proposing an alternative approach for prolonged localized production and delivery of therapeutic proteins in the physiological range through genetic manipulation of cells and tissues. This approach is currently being tested to deliver erythropoietin (EPO) to stimulate red blood cell production. Using a gene therapy approach, the Transduced Autologous Restorative Gene Therapy (TARGT) System utilizes *ex vivo* transduction of dermal tissue fibroblasts (Brill-Almon et al., 2005; Mitrani et al., 2011). A small biopsy of dermal tissue is harvested, cultured in organ culture, and transduced *ex vivo* using a

Abbreviations: BBB, blood brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; EPO, erythropoietin; MO, micro organ; TARGT, transduced autologous restorative gene therapy

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helper-dependent adenoviral-*HuEPO* vector. Previous studies have subcutaneously implanted human EPO-secreting TARGTs ($TARGT_{EPO}$) in immunodeficient mice (Shapir et al., 2015). These TARGTs maintained viability, were well integrated into surrounding tissue, and demonstrated long-term production of EPO in peripheral blood. Clinical trials have been successfully completed in which autologous $TARGT_{EPO}$ was evaluated in patients with anemia associated with chronic renal failure and end stage renal disease (Shapir et al., 2015; Blum et al., 2017).

We conducted a proof-of-concept study with this $TARGT_{EPO}$ platform to demonstrate the promise of delivering sustained levels of therapeutic proteins directly into the CNS. This approach addresses the need for an implanted or refillable reservoir and repeated administration, since the TARGT continually secretes the therapeutic agent *in situ*. Beyond a concept demonstration, EPO also has significance as it acts as a neuroprotective and neurorestorative agent in the CNS (Sargin et al., 2010) and can cross the BBB to achieve clinically effective concentrations in the brain (Merelli et al., 2013). Targeted EPO delivery in the CNS has considerable advantages. Therapeutic EPO concentrations in the CNS delivered systemically would require systemic doses of EPO sufficiently high to stimulate erythropoiesis, which could lead to thromboembolic complications (Ponce et al., 2013).

The goals of these initial experiments were to assess cryopreserved $TARGT_{EPO}$ viability and function after implantation into the rat cisterna magna and to evaluate the immune response of the rat CNS to the implanted TARGTs and secreted hEPO. An early objective was to develop a surgical model that maintained TARGT tissue viability when implanted in the cerebral spinal fluid (CSF) of the brain; the protein, glucose, and oxygen concentrations in CSF are significantly lower than concentrations in peripheral blood. When a TARGT system is implanted into the subcutaneous space, its viability is supported by the rich vascularization of this environment. A maximum 2 × 2 mm TARGT size was selected to allow detection of secreted EPO levels in the CSF and to avoid impeding CSF flow.

Several implantation sites were explored, including the lateral ventricle and the cisterna magna. The cisterna magna was ultimately chosen based on the following rationale: 1) the site allows CSF to more freely flow around the TARGT compared to a convexity site, reducing the likelihood of induced hydrocephalus (Fig. 1); 2) the TARGT can be well visualized throughout the implantation process into the cisterna magna; and 3) the site can be closed using a muscle flap, which possibly provides protection and metabolic support to the TARGT.

2. Materials and methods

2.1. TARGTs and MOs

Rat dermal tissue was harvested from euthanized Lewis rats (LEW/SsNHsd, ENVIGO) using dermatomes (Nouvag NG Ref 1990 and 1992, Goldach, Switzerland). Using a custom built multi-scalpel device, the tissue was cut to the desired dimensions (2 × 2 mm) to accommodate implantation into the rat cisterna magna; these tissues, termed micro organs (MOs), served as controls. Rat MOs were processed *ex vivo* by Medgenics Medical Israel, Ltd. (Misgav, Israel) into erythropoietin-secreting TARGTs ($TARGT_{EPO}$); the MOs were transduced with a Helper Dependent (HD) Adenoviral (Ad) vector encoding human erythropoietin (*HDAd-HuEPO*) (Shapir et al., 2015) at an optimized titer of 1×10^{11} virus particles/TARGT (unpublished data). The resulting TARGTs and MOs were cryopreserved in cryopreservation medium (Thermo Fisher Scientific, Waltham, MA, catalog# A1254201) for shipment and were stored on liquid nitrogen upon receipt.

When ready to be analyzed and implanted, the TARGTs and MOs were thawed using a protocol provided by Medgenics. A cryogenic vial with tissue was immersed into a 37 °C water bath for 1 min, then the contents were mixed with pre-warmed culture medium (HyClone DMEM/F-12 (Thermo Fisher Scientific, Waltham, MA, catalog# SH3002301), supplemented with 50% heat-inactivated mixed-gender Lewis rat serum (BioreclamationIVT, Westbury, NY, catalog# RATSRM-LEWIS) and Antibiotic-Antimycotic 100×, (Life Technologies, Carlsbad, CA, catalog# 15240)), and swirled gently for 2 min. The tissue was transferred into fresh pre-warmed medium supplemented with 50% Lewis rat serum and incubated at 32 °C and 5% CO_2 for 4 h. After that, the tissue was transferred into medium supplemented with 20% Lewis rat serum and incubated at 32 °C and 5% CO_2 for 20 h. Next, the tissue was transferred into medium supplemented with 10% Lewis rat serum, and the culture was continued at 32 °C and 5% CO_2. Medium was collected and replaced every 3 d, for up to 28 d culture. Culture at 32 °C was previously found to maintain higher tissue viability for longer duration and to support higher EPO expression than culture at 37 °C (unpublished data). Collected medium was stored at −20 °C until ready to be analyzed for hEPO levels.

2.2. hEPO secretion from TARGTs and TARGT selection for implantation

TARGTs were placed in implant groups so that the average hEPO expression was similar in all groups. All TARGTs (n = 18) and control MOs (n = 3) were thawed at the same time, and the hEPO secretion level for each TARGT in culture was measured at 3 and 7 days post thaw. The TARGTs were each assigned to one of three implant groups (n = 5 per group) so that the average hEPO level of each implant group was similar to the hEPO expression of all thawed TARGTs. TARGTs were implanted 8 days post thaw as described below. The culture of the remaining TARGTs (n = 3) continued *in vitro,* and the hEPO secretion levels were measured on 14, 21, and 28 days post thaw. hEPO levels in culture medium were quantified with the Quantikine IVD Human EPO ELISA kit (R & D Systems Inc, Minneapolis, MN, catalog# DEP00), according to manufacturer's instructions.

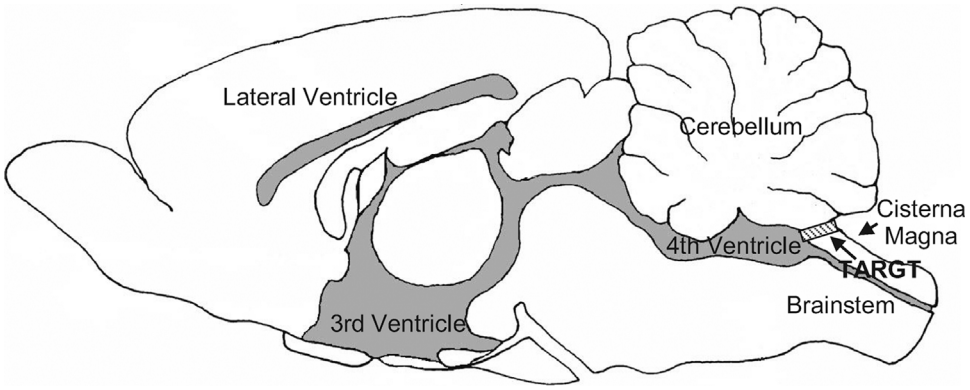

Fig. 1. Schematic of midsagittal section of the adult rat brain. CSF is produced in the ventricles and flows through the spaces shown in gray. Implanted through the cisterna magna (skull missing), a TARGT (hatched) sits between the cerebellum and the brain stem and is in contact with CSF flow.